

DIAGNOSIS OF ORF IN WEST AFRICAN DWARF GOATS IN UYO, AKWA IBOM STATE, NIGERIA

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Abstract

Background: Sixty (60) male West African Dwarf goats were reported with clinical signs of enlarged lymph nodes, scabs on the mouth, nose and ears. Two of the goats died and post mortem examination reveals enlarged submandibular lymph nodes and vesicular lesions on the tongue. Clinical diagnosis of Orf has been reported in Nigeria but this report is the confirmatory diagnosis of Orf in a suspected outbreak in an experimental farm in Uyo, Akwa Ibom State, Nigeria using molecular techniques.

Materials and Methods: Scabs, spleen and lymph node samples from goats suspected to have died from Orf were collected, transported on ice to the laboratory and homogenized. The DNA was extracted using QIAmp DNA minikit (Qiagen) according to the manufacturer's instructions. Orf virus (ORFV) was amplified using published ORFV specific primers by PCR.

Results: Morbidity and mortality were 100% and 3.3% respectively, while ORFV was detected by PCR. Diagnosis of Orf was confirmed based on clinical signs of enlarged lymph nodes, scabs on the mouth, nose and ears, necropsy findings of enlarged submandibular lymph nodes and vesicular lesions on the tongue and PCR results.

Conclusion: This may be the first report of molecular diagnosis of Orf in Nigeria. The 100% morbidity and 3.3% mortality rate is higher than previously reported thus Orf is becoming of greater economic importance than previously thought. It is therefore recommended that routine laboratory diagnosis of Orf be carried nationwide to determine the prevalence of Orf in Nigeria.

Key Words: Orf, goat, PCR, Akwa Ibom, Nigeria

Introduction

Orf, otherwise known as Contagious Ecthyma, is an acute, contagious, debilitating and economically important zoonotic viral skin disease of sheep, goats and wild ruminants. It is caused by Orf virus (ORFV) (Gelaye *et al.*, 2016). Orf virus belongs to the Genus *Parapoxvirus*, subfamily *Chordopoxvirinae*, family *Poxviridae* (Fleming *et al.*, 2015). Orf is also known as sore mouth, contagious pustular dermatitis or scabby mouth, infectious labial dermatitis and it is usually more severe in goats than in sheep. Other animals affected by this virus include alpacas, camels, reindeer, deer, prong horn antelope, wapiti and seal squirrels (Nandi *et al.*, 2011). Orf occurs worldwide and has been reported mostly in late summer, fall and winter on pasture and in feedlots, and the virus can survive for months or even years in suitable environment, with transmission of the disease usually through contact from infected to susceptible animals (Nandi *et al.*, 2011). Orf can be clinically diagnosed based on characteristic lesions on the mouth, legs and perineal region, while confirmatory laboratory diagnosis can be done using electron microscopy, serological tests such as Agar Gel Precipitation Test, Enzyme Linked Immunosorbent Assays (ELISA), Virus Neutralization Test (VNT), histopathology of affected tissues, nucleic acid based assays including Polymerase Chain Reaction (PCR) (Mahmoud *et al.* 2010; Nandi *et al.*, 2010). In Nigeria, there are documented outbreaks of Orf (Obi, and Gibbs 1978; Okoh, 1980; Adeoye, 1984; Obi, 1984; Adah *et al.*, 2012; Kumar *et al.*, 2015). Suspected outbreaks of Orf are frequently reported in Nigeria, but diagnosis is usually done based on clinical presentations without laboratory tests, hence the status of Orf in Nigeria is unknown. The aim of this study was to carry out the confirmatory diagnosis of Orf using molecular technique in a farm from suspected outbreak in Uyo, Akwa Ibom State, Nigeria.

Materials and Methods

Case history

An experimental farm in Uyo, Akwa Ibom State, Nigeria, in July 2014 reported skin lesions around the mouth, nose and ears in their flock of goats, all the goats were affected. History revealed that the farm had 60 male West African Dwarf goats, all of which were bought from local livestock markets and kept under intensive management system. Clinical signs observed were enlarged lymph nodes, scabs on the mouth, nose and ears (Figure 1 and 2). All the animals were affected and the farm recorded two deaths (3.3%). Necropsy findings include enlarged submandibular lymph nodes and vesicular lesions on the tongue.

Sample collection

Scabs were collected from sick animals, while scabs, spleen and lymph nodes were collected from dead animals at necropsy, placed on ice and transported to the Viral Skin Disease Laboratory, National Veterinary Research Institute, Vom, and preserved at -20 °C until used.



Figure 1: Proliferative lesions of Orf virus infections around the lips of a West African Dwarf goat indicated by arrow.



Figure 2: Scab lesions of Orf virus infections around the oral commissures of a West African Dwarf goat shown by arrow head

DNA extraction for Polymerase Chain Reaction

DNA was extracted from the tissue homogenate using QIAamp® DNA mini extraction (QIAGEN, Germany) following the manufacturer's instruction. The DNA was kept at -20 °C until used.

Polymerase Chain Reaction

ORFV specific primers were used as published by Torfason and Gunadottir, (2002) and synthesized by Inqaba Biotec South Africa. The Orf sequence used to design the primer is a 5261bp sequence with an Accession number U33419. This gene encodes RPO132 and it has similar characteristics with the A24R gene of vaccinia (Moss,1996). The ORFV primer sequences are as follows: Orf1: CGCAGACGTGGCTGAGTACGT, Orf2: TGAGCTGGTTGGCGCTGTCCT. The positive samples were amplified at 140 bps.

Orf virus vaccine strain (Orf freeze-dried vaccine, Onderstepoort Biological Products Ltd., Onderstepoort, South Africa) was used as a positive control, and thus subjected to DNA extraction and PCR, while an aliquot of molecular grade water was used as the negative control. The PCR reaction mix comprised of 50µl of 10µl 5X Phusion HF buffer, 1 µl of 10mM dNTPs, 10pmol each of the forward and reverse primers, 0.5µl of Phusion DNA polymerase, 1.5µl of DMSO, 5µl of DNA template, and make the volume up to 50µl using nuclease free water. The cycling conditions for the reaction in a PCR thermal cycler (Geneamp, Applied Biosystem) consisted of an initial denaturation for 12 minutes at 94 °C, followed by 40 cycles of subsequent denaturation at 94 °C for 30 seconds and 68 °C for 45 seconds, after the final extension at 65 °C for 3 min, the samples were held at 4 °C.

Results

Records of the farm revealed that morbidity was 100% (60/60), while mortality rate was 3.3% (2/60). ORFV was detected by PCR, and diagnosis of Orf was confirmed based on clinical signs of enlarged lymph nodes, scabs on the mouth, nose and ears, necropsy findings of enlarged submandibular lymph nodes and vesicular lesions on the tongue and PCR results. DNA extracted from the scabs, lymph nodes, spleen and a positive control were subjected to PCR amplification. Analysis of the amplified products by agar gel electrophoresis showed a single DNA fragment of 140bps (Figure 3).

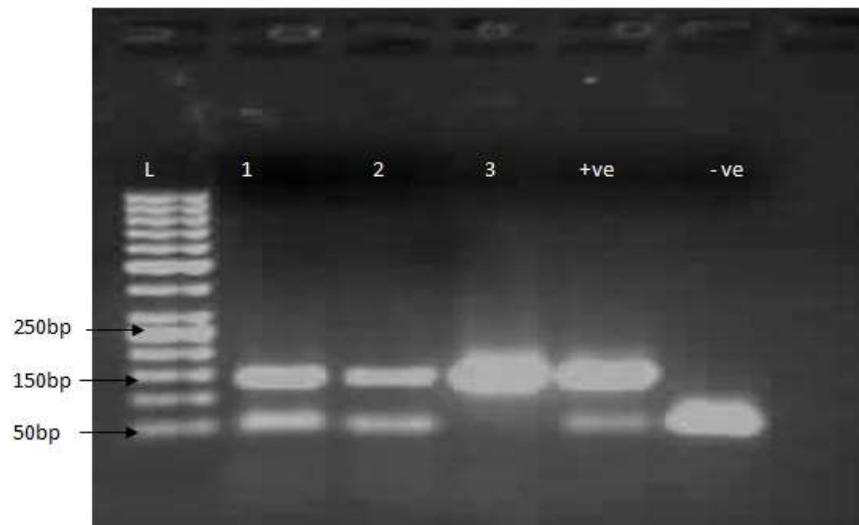


Figure 3: Visualization of polymerase chain reaction product of Orf virus from scab, lymph nodes and spleen of West African Dwarf goat. Lanes 1 is lymph node, Lane 2 is spleen sample and lane 3 is scab samples, +ve is the positive control and -ve is the negative control. Positive samples were amplified at 140 bps and the ladder is 50bps

Discussion

Sheep and goats are very important and integral part of livelihood in rural areas and in most parts of the world where they are used as banks by rural dwellers (Kumar *et al.*, 2015). Orf has been reported to be an important and endemic disease of goats in southern parts of Nigeria with seroprevalence of 61% (Adeoye, 1984; Obi, 1984). The morbidity rate in the present study was 100%, and such high morbidity rates are common in outbreaks of Orf (Nandi *et al.*, 2011; Scagliarini

et al., 2012). However, in this report the mortality rate was 3.3%, which is higher than previous mortality rate of 2.6% reported in goats in southwestern Nigeria (Adeoye, 1984). Although ORFV infections are self-limiting with low mortality rates, mortality rates of 20-90% have been reported in severe outbreaks (Scagliarini *et al.*, 2012). The proliferative lesions around the mouth observed in this report are characteristic clinical presentation of Orf, which is not confirmatory, because such lesions are also seen in outbreaks of Peste des petits ruminants, Sheep pox (SPP) and goat pox (GTP) (Nandi *et al.*, 2011; Gelaye *et al.*, 2016). In this study, ORFV was detected by PCR in the scab, lymph node and spleen samples collected during the Orf outbreak which confirms the diagnosis of the disease. Diagnosis of Orf in Nigeria is usually based on clinical presentation and it is rarely reported, probably because Orf is neither a controlled nor notifiable disease, therefore the prevalence and the economic impact of the disease is unknown or underestimated. Orf virus is highly contagious, zoonotic and the disease is an occupational hazard to veterinarians, shepherds and animal handlers (Nandi *et al.*, 2011, Scagliarini *et al.*, 2012). Small ruminants like goats are an integral part of the lives of rural populace in Nigeria and they serve as a source of food and income for this segment of the population. Hence, the prevention and progressive control of Orf will help in the growth and preservation of the population of goats in Nigeria. Small ruminants generally are source of income of rural smallholders' farmers who depend on them for money during times of need. Prevention of Orf in goats will help alleviate poverty in this segment of the population of the rural poor.

Conclusions

This is a confirmed case of Orf based on the detection of the ORFV using specific primers by PCR and the characteristic Orf lesions observed. This is the first report of molecular diagnosis of Orf in Nigeria. The 100% morbidity and 3.3% mortality rate reported in this study are respectively higher than those previously reported (Adeoye, 1984). Thus, Orf is becoming a greater 'economic burden' than previously thought. It is, therefore, recommended that routine laboratory diagnosis of Orf should be carried out to determine the prevalence and economic impact of Orf on livestock production in Nigeria.

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References

1. Adeoye, S.A.O. (1984). Disease profiles of sheep and goats in two groups of villages in southwest Nigeria. Sheep and Goats in Humid West Africa. Proceedings of the Workshop on Small Ruminant Production Systems in the Humid Zone of West Africa, Ibadan, Nigeria, 23 - 26.
2. Adah, B. M. J. Olabode H. O. K. and Ajayi I. E. (2012). A Case of Contagious Pustular Dermatitis in Kano Brown Goats. Sahel Journal of Veterinary Science, 11 (2) 23 – 27
3. Fleming, S.B., Wise, L., N and Mercer, A.A. (2015). Molecular genetic analysis of orf virus: a poxvirus that has adapted to skin. Viruses, 7: 1505-1539.
4. Gelaye E, Achenbach J.E., Jenberie, S., Ayelet, G., Belay, A., Yami, M., Loitsch, A., Grabherr, R., Diallo, A. and Lamien, C.E. (2016). Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008–2013. Virology Journal, 3 (34): 1-12
5. Kumar R., Narayan, R., Bhatt, P., Haq, S. Khurana, S.K., Tiwari, R., Karthik, K., Malik, Y.S., Dhama, K., Chandra, R. (2015) Contagious pustular dermatitis (Orf Disease) – epidemiology, diagnosis, control and public health concerns. Advances in Animal and Veterinary Science, 3(12): 649-676
6. Mahmoud, M, Abdelrahman, K. and Soliman, H. (2010). Molecular and virological studies on contagious pustular dermatitis isolates from Egyptian sheep and goats. Research in Veterinary Science, 89: 290–294.
7. Moss B. (1996). Poxviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, et al., editors. Fields virology, vol. 2, 3rd ed. Philadelphia: Lippincott–Raven Publishers, 2637–71.
8. Nandi, S. Ujjwal K., D. and Chowdhury, S. (2011) Current status of contagious ecthyma or Orf disease in goat and sheep—A global perspective. Small Ruminant Research, 96: 73–82
9. Obi T U and Gibbs, E. P. J. (1978). Orf on sheep and goats in Nigeria. Tropical Animal. Health Production, 10: 233-235.

10. Obi, T. U. (1984). Serological survey of some viral infections in goats in Southern Nigeria. In: Sumberg, J. E. and Cassaday, K. (eds). Sheep and Goats in Humid West Africa. Proceedings of the Workshop on Small Ruminant Production Systems in the Humid Zone of West Africa, Ibadan, Nigeria, 23 - 26 January, 1984.
11. Okoh, A. E. J. (1980). Contagious Ecthyma in exotic sheep in Nigeria, *Tropical Animal Health Production*, 12:192.
12. Scagliarini, A., Piovesana, S., Turrini, F., Savini, F., Sithole, F. and McCrindle, C.M. (2012). Orf in South Africa: Endemic but neglected. *Onderstepoort Journal of Veterinary Research*, 79(1): 488
13. Torfason, E. G. and Guonadottir, S. (2002). Polymerase chain reaction for laboratory diagnosis of Orf virus infections. *Journal of Clinical Virology*, 24: 79–84.